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Exploring Tensile Mechanics of Cribellar Hypochilus pococki

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Abstract

The two major types of silk seen, are cribellate and viscid silk. Cribellate silk is produced by the behavioral trait of combing the silk with the spiders hindlegs to achieve the adhesion property needed to capture prey in a web. This study aims to classify the most primitive cribellate capture thread, from a species not studied thus far- *Hypochilus pococki.* Previous studies on cribellate silk, have produced extraordinary tensile results, increasing the need to study more cribellate species (Michalik et al, 2019). The results found suggest that *Hypochilus pococki*, does not exhibit extreme values of tensile mechanics, but more of an average value when compared to other cribellate species. *Hypochilus pococki* is one of the most primitive cribellate species around today, so the results further our understanding of common ancestral tensile mechanics among all spiders.

Introduction

Cribellate spiders are a unique set of spiders that use an ancestral type of adhesive for the capture portion of their web (Blackledge & Hayashi, 2006). Cribellate spiders achieve this because they possess an additional organ, called the cribellum. The cribellum is a plate covered in thousands of spigots. From these spigots, very thin threads are fabricated from the physical act of combing. Threads are combed by the calamistrum. The calamistrum consists of bristled hairs with tiny teeth, located on the hind legs of the spider. The act of combing the thread, gives the thread its adhesive property. This combed thread is used for the capture thread of the web (Foelix 2010).

The core fiber of cribellate silk is psuedoflagelliform, surrounded by axial fibers, reserve warps and nano fibers. The axial fibers are surrounded by puffs of cribellar fibrils (Michalik et al, 2019). The fibrils use van der Waals and hygroscopic forces to allow for adhesion (Hawthorn & Opell, 2002).

Cribellate spiders differ from the typical viscid spider. Viscid silk is thought to have evolved after cribellate silk and the orb web architecture (Blackledge 2006). Viscid spiders do not possess a cribellum, instead they obtain adhesion through the form of an aqueous glue. The glue is composed of glycoproteins, that create nanosprings that offer extensibility to the web (Blackledge & Hayashi 2006). Many studies have been conducted on viscid silk and its extensibility, but only some research has been done on cribellate spiders to generalize their extensibility as a family.

In a previous study, samples from 3 spiders in the genera Deinopoidea were tested, this included two different genus, Deinopidea and Uloboridea. It is important to sample across genus to compare differences, because there are many variables, such as web architecture that could factor in to results. It is also important to sample different genus to be able to draw a generalized conclusion of tensile properties. Four species were used, *Deinopis spinosa, Hyptiotes cavatus, Hyptiotes gertschi* and *Uloborus diversus. Uloborus* and *Hyptiotes,*are unique in that the lack a reserve warp. This study found that cribellate silk's properties were unique when compared to viscid capture threads. When tested, it was observed that the axial fibers of cribellate silk were only stretched to 50-100% their original length after failure of the axial thread occurred. After the axial fiber failed, cribellar fibrils absorbed the energy and allowed the thread to continue to stretch to as much as 500% the original length, until complete failure of the thread. Among the four species studied in this study, axial extensibility, total extensibility, and work by cribellar fibrils differed between species. Overall, this study showed that mechanical tensile property of cribellate capture thread depends on the core axial fiber and also on the hundreds of fibrils in the cribellar fibrils. Although differences remain between species in energy absorption and extensibility values as a percentage of their original length (Blackledge & Hayashi, 2006). A different study of cribellate capture threads was conducted, using three different species, *Octonoba sinensis, Ulboborus glomosus,* and *Waitkera waitakerensis.* This study found extensibility values ranging from 299-753% of the original silk length (Opell & Bond, 2000).

Very different results were found in another study on cribellate spiders that involved a different species, *Progradungula otwyrensis,* not studied previously. The combed cribellate threads were found to extend over 1,400% their original length. This value is higher than any other spider capture silk. This is likely due to the composition of the thread, consisting of a tightly coiled reserve warp, that gradually uncoiled when stretched, leading to a high value of extensibility (Michalik et al, 2019). This result differs significantly from what was previously found on cribellate spiders, and could be due to the extreme coil of the reserve warp that is observed in *Progradungula otwayensis.* Especially, since it was observed that *Uloborus* and *Hyptiotes* lack a reserve warp. This finding is very important, and suggests that more research must be done to generalize cribellate tensile properties. This result raises a new question, is the extensibility value found in *Progradungula otwayensis,* unique to that species, or do more cribellate species also possess this extreme value of extensibility?

Understanding the differences in extensibility between different genera of cribellate species will increase knowledge on ancestral traits of all spiders. To understand a spiders extensibility, an analysis of extension verses load needs to be conducted. This study will analyze the tensile properties of a cribellate species, *Hypochilus pococki. Hypochilus pococki* is thought to belong to the category of the most primitive cribellate spiders. Thus, *Hypochilus pococki* is particularly interesting to study to get a better understanding of the common ancestor of cribellate spiders in comparison to the unique extensibility observed in *P. otwayensis.* Due to the evolutionary position of *Hypochilus pococki,* it is hypothesized that this species will have more basic extensibility characteristics, when compared to *P. otwayensis.*

Materials and Methods

Hypochilus pococki is a member of the hypochilidae family, and is distinguished by their particular style of web building, a unique lamp shade style. Samples were collected in the northern part of the Appalachian mountains in the United States. They prefer dark and moist environments, often building their webs in caves. Cages were constructed to resemble their natural cave-like setting. Cardboard pieces were added to allow the spider to add web attachments at variable lengths. Spiders were observed to only spin webs after an element of humidity was added to their environment. Humidity was obtained through misting of the cages regularly with tap water, and adding a source of water in a weigh boat. Spiders were fed small crickets once a week. A total of three spiders spun webs in captivity. Spider one spun five webs, spider two spun one web, and spider three spun three webs. Due to the low number of webs, silk from all spiders was combined for analysis.

Due to the lamp shade style of the web, individual capture threads were difficult to collect. First the spider was removed from the web in order to minimize scaring the spider. If the spider was scared in the process, it would run through the web, damaging that portion of the web, so it was important to do this carefully. A circular wire loop covered in double sided tape was used to collect a portion of the web. Collection directly from the web was difficult due to how tiny the individual silk threads were. Shorter collection samples allowed for a more accurate collection of the cribellate silk. A soldering iron was used to burn off the edges of the threads around the circular loop, so no pulling of the web would occur to affect results. The loop was observed under a compound microscope and cribellar threads were identified by their composite of axial threads and coiled reserve warps. Cribellate threads were differentiated from radial threads by their glistening appearance due to the adhesive nanofibrils. Individual samples were collected across 6.8 mm gaps in cardboard mounts. Silk was secured to the carboard with superglue, to prevent the silk from moving or stretching after it was collected.

Tensile properties were found by running tensile tests on a Nano Bionix tensile tester. Tensile tests were run with a strain rate of 0.01 1/s, a max strain of 5.0, and a nominal gauge length of 6.0 mm. Due to the nature of the silk (multiple fibers in a sample and nanofibrils obscuring the interior fibers during SEM), diameters were too difficult to measure, so force data was not normalized to cross-sectional area. Extensibility was measured for both portions of the test, axial and warp fiber. The axial portion was identified by the extension of the axial fibers, with a steep slope and a noticeable break in the thread. Once the axial fibers broke, the warp fiber portion of the test was identified, as a slower unwinding of the warp fiber threads, until an eventual break of the entire thread. Extension axial and warp fiber values were added together, and then calculated as a percent change in length.

Results

The cribellate silk from *Hypochilus pococki* exhibited an initial elastic behavior in the axial threads composing the silk, until a yield was reached, and the axial threads failed. After the axial thread failed, a slow uncoiling of the reserve warp was observed, adding to the silks overall extensibility. Once, the warp fiber threads were maximally extended, the thread failed. This can be seen in figure 1.

Figure 1

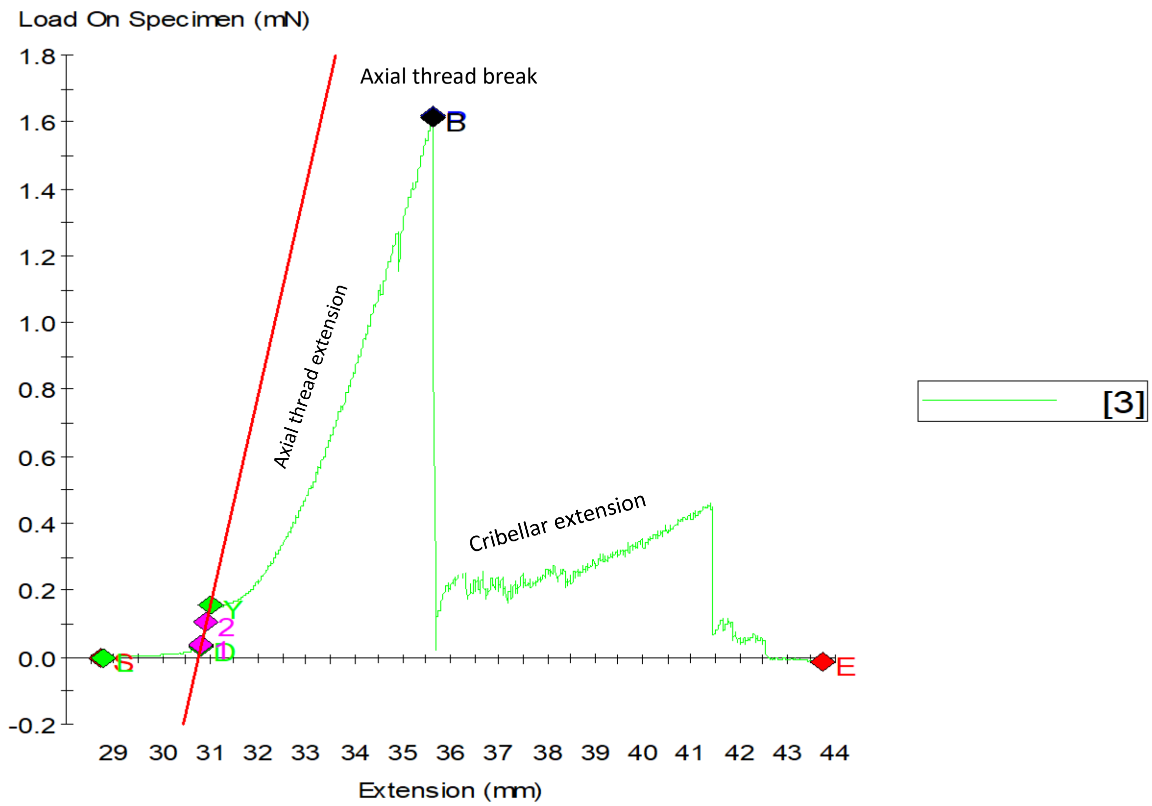

Fig 1. Tensile test of *Hypochilus* silk, generated on a load (mN) verse extension (mm) graph. Axial thread extension was observed initially, until failure around 1.7 mN load. After axial thread failure, warp fiber thread extension was observed, with a decrease in slope, and more slowly extended, until total failure of the thread was observed.

Extensibility was measured for the axial portion of the test, the warp fiber portion, and then as a sum of the two, for total extensibility of the thread. Axial extensibility was found to be 97.7% of the original length of the thread. Followed by warp fiber extension with 79.8% extension. When adding the two value together, total extensibility of the silk thread was calculated to be 177.5% of the original length. Table 1 shows a comparison of these 3 values.

Table 1

Portion of Test	Extension (%)
Axial	97.7
Warp Fiber	79.8
Total	177.5

Table 1. Comparison of percent extensibilities between the 2 different portions of the test, and total extensibility of the entire thread.

The average total extensibility for all samples tested was calculated to be 177.5% of the original length of the silk. Table 2 shows a comparison of this value, to other known values of cribellate spiders, previously studied.

Table 2

Spider	Extensibility(%)
P. otwayensis	1443
Octonoba sinensis	753
Uloborus glomosus	604
Deinopis spinosa	511
Waitkera waitakerensis	299
Hypochilus pococki	177.5
Uloborus diversus	159
Hyptiotes cavatus	118
Hyptiotes gertschi	88

Table 1. Comparison of percent extensibility values among cribellate spiders (Michalik, et al, 2019).

An analysis of the three spiders individual data was conducted to observe differences between the three spiders silk. Table 3 shows the differences between axial, warp fiber and total extensibility. The data shows that spider one has a significantly higher warp fiber extensibility, contributing to a higher total extensibility.

Table 3

Extension (%)	Spider 1	Spider 2	Spider 3
Axial	96.9	115.0	97.6
Warp Fiber	96.0	13.0	19.4
Total	192.9	128.0	117.1

Table 3. A comparison of the three spiders used.

Discussion

Cribellate spiders exhibit a unique behavioral process of combing their silk to achieve the adhesion property of the capture silk in the web. Cribellate spiders are believed to be ancestral to all spiders today, with the viscid spiders evolving from the cribellate spider. The data found in this study, explores a species not studied before, to give further insight into generalizing cribellate silk tensile properties. This species, *Hypochilus pococki,* is particularly interesting because it is one of the most ancient cribellate spiders. Data on *Hypochilus pococki,* will give a better understanding of ancestral cribellate silk, and how it has evolved between species.

The total extensibility percent value found, was 177.5% of the original silk length. The silk achieved this extensibility due to its components, axial fibers, reserve warps, and cribellar puffs. In comparison to other cribellate species studied, the data suggests

Hypochilus pockocki to be on the lower spectrum of extensibility. *Hypochilus pococki* was most similar to *Uloborus diversus.* A big difference was observed between *Hypochilus pococki and Progradungula otwayensis,* with *Progradungula otwayensis* exhibiting extreme tensile properties, unlike any spider species. I hypothesize that all cribellate spiders had a similar extensibility to that of *Hypochilus pococki,* and evolved from there, leading to the major differences seen in table 2. *Progradungula otwayenis,* uses a different web building technique than *Hypochilus pococki*, using a catching ladder structure. Their cribellate silk is composed of highly coiled reserve warps and puffs of nanofibers. The extreme coil seen in their reserve warp, is what provides the high extensibility seen in their silk. This was a very important finding, as it suggests that further studies into different cribellate spiders should be done, to see if any other cribellate species share this characteristic.

After analyzing the three spiders individually and comparing their data, a significant difference was seen in spider one's warp fiber extension, compared to spiders two and three. Spider one had a warp fiber extensibility of 96.0%, this was very close to spider's one axial thread extensibility. Spider two and three differed in this, with values of 13.0% and 19.4%, for warp fiber extensibility, respectively. This difference could be due to spider one being collected in June, and spiders two and three being collected in September. Spider one immediately spun webs once in captivity, while spiders two and three did not spin webs until a month of being in captivity. The overall appearance of the webs and silk was similar among the spiders, but small differences existed. Spiders two and three spun tighter webs, leaving less room in between silk threads, and thus, making it harder to collect.

I propose that more studies on *Hypochilus pococki* tensile mechanics should be conducted, with more spiders and silk samples, in order to draw more accurate results to generalize the extensibility value for the entire species. Further studies should also explore other cribellate spiders not studied yet, to increase knowledge on the properties of cribellate silk and its possible future implications into biomechanics and medicine.

References

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